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SCREENING FOR AMYLYOLYTIC AND BACTERIOCINOGENIC LACTIC ACID BACTERIA FROM CURD, MILK AND SOIL

Microbiology

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ABSTRACT

In fermentation, bacteriocinogenic and amylolytic properties of lactic acid have been reported to be important functional properties. Lactic acid producing bacteria (*Lactobacillus* sp.) from milk and traditionally fermented food were screened for amylyolytic and bacteriocinogenic properties. Amylyolytic strain was determined by starch hydrolysis test while Antagonistic activity against several spoilage organisms were determined by lawn culture and bacteriocinogenic property was evaluated using agar well diffusion test after elimination of lactic acid and hydrogen peroxide. Lactic acid and Hydrogen peroxide were eliminated because they have antagonistic property just like bacteriocin hence undesirable. A total of nineteen colonies were picked from various samples (Curd, Condensed milk, Raw milk and Idli) and were subjected to bacteriocin assay and amylase test. All the strains showing relative positive results, were selected and subjected for further characterization. The numbers of *Lactobacillus* strains that have amylyolytic property and those that have bacteriocin property were noted. Among all the isolates bacteriocin activity was found in P1, R3, R6, R7, R8 & T7 strains against various test organisms and P1, R3, R6 shows amylolytic property. The isolate showing the two properties are good candidate for further research in usage as starter culture for fermentation.

KEYWORDS

Bacteriocin, Starch Hydrolysis, Fermentation, LAB

INTRODUCTION

The **lactic acid bacteria (LAB)** comprise of Gram positive, acid-tolerant, generally non-sporulating, non-respiring rod or cocci. They are usually found in decomposing plants and lactic products and produces lactic acid as the major metabolic end-product of carbohydrate fermentation. Lactic acid and other metabolic products contribute to the organoleptic and textural profile of a food item (*Ben Omar et. al. 2008*). LAB produce large amounts of lactic acid, which prevents the growth of spoilage microorganisms. Moreover, some strains of LAB produce bacteriocins, which also inhibit the growth of spoilage bacteria in fermented foods. Bacteriocins from LAB have attracted special interest from the standpoint of their potential use as safe and natural food preservatives (biopreservatives) and antimicrobials (*Cleveland et al., 2001*)

Lactic acid bacteria produce a variety of antagonistic factors that include metabolic end products, antibiotic-like substances and bactericidal proteins, termed bacteriocins. The range of inhibitory activity by bacteriocins of lactic acid bacteria can be either narrow; inhibiting only those strains that are closely related to the producer organism, or wide; inhibiting a diverse group of Gram-positive microorganisms. Moreover, Amylolytic lactic acid bacteria, which can convert starch directly to lactic acid, are desirable in fermentation (*Reddy et al., 2008*). The use of amylolytic lactic acid bacteria provides an alternative by combining amylase production and acidification by using the same strain, which could improve control of fermentation and lead to product of more regular quality (Songre-Ouattar *et al*., 2009).

MATERIALAND METHODS

Collection of samples.

Curds, Milk, Idli – a locally fermented food in India and soil sample were collected in sterile containers and transported to the laboratory of Helix Biogenesis, NOIDA for analysis within 24 hours.

Isolation Procedures

Ten grams each of Idli and soil samples were serially diluted in 100ml of sterile distilled water and homogenized while 10ml of curd and milk samples each were serially diluted in 90ml of sterile distil water. Serial dilution was carried out on respective samples to obtain dilution factor of 10^{-6}. One ml of appropriate serial dilution were plated with molten MRS agar plates and incubated anaerobically at 30˚ C for 48 hours. (*Harigan and McCane, 1976)*

After incubation, plates were observed for bacterial growth and distinct colonies were randomly selected. Selected colonies were picked and repeatedly streaked on MRS agar plates until pure cultures were obtained.

The pure cultures were maintained on MRS agar plate at 5˚ C after visible growth on the plate following incubation at 30˚ C.

BACTERIOCIN ASSAY

Preparation of a Cell free Culture Supernatant

All the isolated strains were tested for bacteriocin production by propagating in MRS broth (pH 5.5) for 72 hours at 30˚ C in an anaerobic jar. Cell free solutions were obtained by centrifuging the broth culture at 3000 rpm for 15 minutes and the supernatant decanted into a new eppendorf tube followed by another centrifugation until a pure supernatant was gotten. It should be noted that the supernatant could be filtered through a 0.2 micrometer pore size cellulose acetate filter in other to get a purer form and concentrated 10 folds with a rotary evaporator. It is then adjusted to pH 6.5 with sterile NaOH (1M) and inhibitory activity from hydrogen peroxide is eliminated by the addition of 5mg/ml catalase to the supernatant.

Indicator Organisms

The indicator organisms (*Staphylococcus aureus, Escherichia coli, Pseudomonas, Bacillus*, were obtained from the Institute of Microbial Technology based in Chandigarrh. They were maintained on nutrient agar slants at 5˚ C and transferred at two weeks intervals.

Screening LAB for Amylase Production Potential (screening for amylolytic activity).

STARCH HYDROLYSIS.

Determination of amylase production potentials of LAB isolates was carried out using starch hydrolysis test following procedures adapted by Sun et al. (*Sun et al., 2010*) LAB isolates were streaked (zig-zag) inoculated onto sterile solidified MRS agar fortified with 2% soluble starch and incubated at 30°C for 24 hours. The plates were then flooded with Gram's iodine (Gram's iodine; 0.15% iodine crystals added to 1.5% potassium iodide solution) to produce a deep blue colored starch-iodine complex. It was then observed for zone of decolorization which becomes visible within few seconds. Bacterial isolates surrounded by clearing zones were then sub-cultured on MRS agar to obtain pure cultures for further analysis.

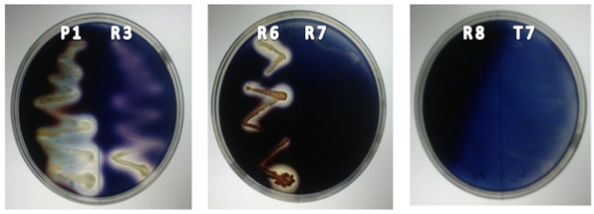
Detection of Antagonistic Activity (Bacteriocinogenic activity).

The antimicrobial activity of LAB isolates was first detected by the agar well diffusion method as described by Schillinger and Lucke (1989). The indicator organisms used were *Staphylococcus aureus, Escherichia coli, Pseudomonas, Bacillus*. 1 ml of the broth culture of each indicator organisms were spread on already set nutrient agar plates. Wells of 5mm in diameter were cut in the agar using sterile cork-borer. A sterile pipette was used to introduce 50 microliter of the cell free supernatant. The plates were left for 2 hours at 5˚ C to allow the broth diffuse into the agar after which they were incubated for 24

hours. The plates were then checked for possible clear zones of inhibition and the diameters were measured.

RESULTS AND DISCUSSION

A total of nineteen colonies were randomly picked from the various samples. They were picked based on distinct colonial characteristics. They were all subjected to bacteriocin assay and the strains showing a relative positive result were used for further characterization. A total of six isolates were selected and subjected to morphological, physiological and biochemical tests.



STARCH HYDROLYSIS Test

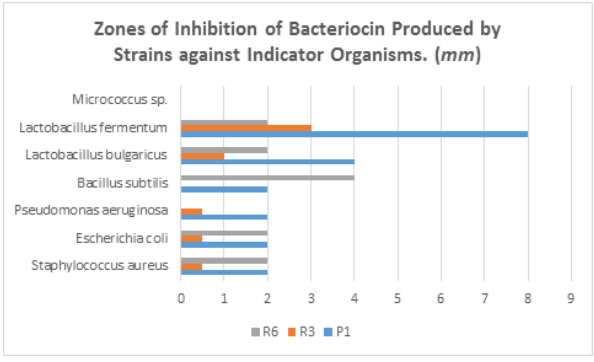
Table 1: Biochemical and physiological characterization of bacteria isolates showing bacteriocin activity

Isolates	Morphology	Gram's Reaction	Catalase	Oxidase	Citrate	Iindole	Metyl Red	V.P	Urease	at pH 4.5	at pH 9.6	4%NaCl	10%NaCl	Homo/Hetero	Dextrose	Lactose	Sucrose	Manitol Agar
P1	rods	+	-	-	-	+	-	-	-	+	+	+	+	HT	+	+	+	-
R3	rods	+	-	-	-	-	-	-	-	-	+	+	+	HM	+	+	+	+
R6	cocci	+	-	+	-	-	-	-	-	+	+	+	+	HT	+	+	+	-
R7	rods	+	-	+	-	-	-	-	-	+	+	+	+	HM	+	-	+	-
R8	rods	-	-	-	-	+	-	-	-	-	+	+	+	HM	+	+	+	-
T7	rods	+	+	-	-	+	+	-	+	-	+	+	+	HM	+	+	+	+

Table : Zones of inhibition of bacteriocin produced by strains against indicator organisms. (mm)

Indicator Organism	P1	R3	R6	R7	R8	T7
Staphylococcus aureus	+(2)	+(0.5)	+(2)	+(2)	+(1)	+(1)
Escherichia coli	+(2)	+(0.5)	+(2)	+(4)	+(3)	+(3)
Pseudomonas aeruginosa	+(2)	+(0.5)	-	+(3)	+(1)	-
Bacillus subtilis	+(2)	-	+(4)	+(4)	+(3)	+(3)
Lactobacillus bulgaricus	+(4)	+(1)	+(2)	+(1)	-	-
Lactobacillus fermentum	+(8)	+(3)	+(2)	+(1)	+(2)	+(2)
Micrococcus sp.	-	-	-	+(4)	-	-

+ means sensitive. **- means resistance.**
5 to 9mm = strong inhibition; **0.5 to 4 = weak inhibition.**



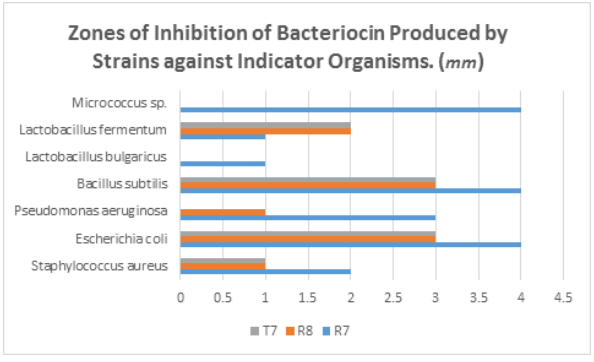


Table: Starch Hydrolysis Test

Isolated Strain	Starch Hydrolysis
P1	+
R3	+
R6	+

While various kinds of LAB bacteriocins have been discovered so far, novel ones will be needed to achieve more effective control of undesirable bacteria. Therefore, various sources have been explored to isolate novel bacteriocin-producing LAB with appropriate features for target foods or infections. Several LAB bacteriocins offer potential applications in food preservation. The use of bacteriocins in the food industry could help to reduce the addition of chemical preservatives as well as the intensity of heat treatments. Also, Numerous attempts have been made to improve fermentation to decrease the production cost of lactic acid. Recently, amylolytic lactic acid bacterial fermentation has been a subject of significant interest because of the cost-effective nature of the starchy substrates, which do not have to be saccharified. However, there have been very few reports on the isolation of amylolytic lactic acid bacteria for single-step fermentation of inexpensive complex carbohydrates to lactic acid. Therefore, this study investigated both bacteriocinogenic and amylolytic lactic acid bacteria.

CONCLUSION

In this study, the isolated microbe showed amylolytic property and bacteriocin production and its effect against different microbial species, these microbes have been isolated from different sources as curd, milk, Idli and soil, although soil isolate do not possess the desired expected characteristic. The selected isolated strain producing bacteriocin and positive for starch hydrolysis have been further characterized. Among all seven isolates P1,R3,R6,R7,R8,T7, Bacteriocin activity were found in P1and R7 strains against various test organisms while P1, R3 and R6 were positive for starch hydrolysis. Best bacteriocin producing microbe strain P1, R3, R6 were the only organism that were positive for bacteriocin production and starch hydrolysis; thus further study on this strain would be required to ascertain their usage as a starter culture strain

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